PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 23.08.2004
Applicant's or agent's file reference AHBCP6047252	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 02/02668	30.05.2002	30.05.2002

Applicant
ASTEX TECHNOLOGY LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Brandt, M Tel. +49 89 2399-2926

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference AHBCP6047252	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 02/02668	International filing date *(day/month/year)* 30.05.2002	Priority date *(day/month/year)* 30.05.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/53		
Applicant ASTEX TECHNOLOGY LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.12.2003	23.08.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Espen, J Telephone No. +49 89 2399-8410

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 02/02668

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-68	as originally filed

Claims, Numbers

1-4, 15 (part), 16-20	as originally filed
5-14, 15 (part)	received on 07.06.2004 with letter of 02.06.2004

Drawings, Sheets

1/1	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-20
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1). The present International Application relates to the isolation and purification of cytochrome P450s. For this purpose a high ionic strength (i.e. a high concentration of salt) is used in an early stage of the recovery process, and provides for recovery of protein in a non-aggregated state.

2.1). The following documents were considered:

D1 (VON WACHENFELDT CLAES ET AL: 'Microsomal P450 2C3 is expressed as a soluble dimer in Escherichia coli following modifications of its N-terminus.' ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 339, no. 1, 1997, pages 107-114, XP002222918 ISSN: 0003-9861 cited in the application)

D2 (WILLIAMS PAMELA A ET AL: 'Mammalian microsomal cytochrome P450 monooxygenase: Structural adaptations for membrane binding and functional diversity.' MOLECULAR CELL., vol. 5, no. 1, January 2000 (2000-01), pages 121-131, XP002222921 ISSN: 1097-2765 cited in the application)

2.2). D1 describes the expression of heterologous (rabbit) P450 2C3. In order to allow a subsequent isolation without the necessity to use detergents, the putative membrane-spanning domain from the N-terminus P450 2C3 was removed, preventing the integration of the modified proteins into *E. coli* membranes.
Moreover, D1 discloses that the subcellular distribution of P450 2C3 in *E. coli* is dependent on the ionic strength of the buffer used for cell disruption (in buffers containing 1 M NaCl or 0.5 M KPi, P450 2C3d was predominately found in the soluble fraction) (D1, abstract). Additionally, the incorporation of four histidine residues at the C-terminus (P450 2C3dH) allowed the extraction of P450 2C3d in the absence of detergent (D1, abstract).
The variant P450 2C3dH and 2C3d are predominantly dimers, whereas 2C3 is a larger oligomer (D1, abstract).
The dissociation of truncated P450s to monomers with detergent prevented effective reconstitution of catalytic activity under conditions where the catalytic activity of the full-

length enzymes was not affected by the presence of the detergent (D1, p. 113).

D2 describes the use of engineered P450s (2C5) to produce diffraction quality crystals that have yielded the first mammalian structure of a microsomal cytochrome. Said P450s comprised mutations (N202H, I207L, S209G, and S219T) which are each sufficient to decrease the aggregation of 2C5dH in high salt from a tetramer to a monomer (D2, p. 129).
Moreover, D2 refers to D1, and states that the D1 P450 preparations were not amenable to crystallization.

3.1). Having regard to the available prior art, the claimed matter is novel (Art. 33 (2) PCT).

3.2). In view of the closest prior art document D2, the claimed matter is also considered as being inventive for the following reasons:

The claimed matter differs from D2, in that in the present application the (truncated) **human** P450 cytochromes were expressed in E. coli, and upon isolation and purification were suitable for crystallization without the need of being mutated in order to reduce aggregation.

Having regard to the above comments, the claimed matter was neither described nor suggested by the available prior art, and therefor, the requirements of Art. 33 (3) PCT are also fulfilled.

3.3). The industrial applicability is acknowledged (Art. 33 (4) PCT).

5. The method of claim 4 wherein step (f) is performed by removing salt from said preparation by size-exclusion chromatography.

6. The method of any one of the preceding claims wherein the P450 carries a polyhistidine tag.

7. The method of any one of the preceding claims wherein the P450 is a member of the CYP1, 2, 3 or 4 family.

8. The method of claim 7 wherein the P450 is a CYP2 family member.

9. The method of claim 8 wherein the P450 is 2C9 or 2C19.

10. The method of claim any one of the preceding claims wherein the P450 comprises a deletion in its N-terminal membrane inserting element.

11. The method of claim 10 wherein the N-terminal sequence of said P450 comprises, in place of the N-terminal membrane inserting element, a sequence MAKKTSSKGR or MAYGTHSHGLFKK.

12. The method of claim 11 wherein said P450 is of SEQ ID NO:2, 4, 6 or 8.

13. The method of any one of the preceding claims which further comprises crystallizing the P450.

14. A crystal of a human cytochrome P450 selected from the group of 2C9, 2C19, 2D6 and 3A4.

15. The crystal of claim 14 wherein said P450 is 2C19 and said crystal has cell dimensions of a=158Å, b=158Å, c=212Å